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EXAMINER	
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Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Office Action Summary	Application No.	Applicant(s)	
	10/813,693	TABOR ET AL.	
	Examiner	Art Unit	
	Angela Bertagna	1637	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE *3* MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *21 September 2007*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1,11,24 and 124-169* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1,11,24 and 124-169* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____.
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

DETAILED ACTION

Status of the Application

1. Applicant's response filed on September 21, 2007 is acknowledged. Claims 1, 11, 24, and 124-169 are currently pending. In the response, Applicant amended claims 1, 11, 124-127, 157, 158, and 160-163.

Applicant's arguments and amendments filed on September 21, 2007 were sufficient to overcome: (1) the objection to the specification, (2) the rejection of claims 1, 11, 124-142, and 147-163 under 35 U.S.C. 112, 2nd paragraph, (3) the rejection of claims 1, 11, 124-128, 130, 131, 137, 138, 141-148, 151, 152, 156-159, 166, and 168 under 35 U.S.C. 102(b) as anticipated by Nakai, and (4) the rejection of claims 24, 124-127, 129, 132-136, 139, 140, 149, 150, 153-155, 157-158, 160-165, 167, and 169 under 35 U.S.C. 103(a) citing Nakai as the primary reference. Accordingly, these rejections have been withdrawn.

This Office Action includes new grounds of rejection not necessitated by Applicant's amendment, and therefore, is made non-final.

Claim Rejections - 35 USC § 112 – 2nd paragraph

2. The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

Claims 143-146 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Claims 143-146 are indefinite because they recite the phrase "less than about". The phrase "less than about" is indefinite, because it is entirely unclear what temperature is "less than about 60° C", "less than about 50° C", "less than about 45° C", and "less than about 40° C." See also MPEP 2173.05(b), which states, "....the court held that claims reciting 'at least about' were invalid for indefiniteness where there was close prior art and there was nothing in the specification, prosecution history, or the prior art to provide any indication as to what range of specific activity is covered by the term 'about.' Amgen, Inc. v. Chugai Pharmaceutical Co., 927 F.2d 1200, 18 USPQ2d 1016 (Fed. Cir. 1991)."

Claim Rejections - 35 USC § 102

3. The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

> A person shall be entitled to a patent unless –
>
> (a) the invention was known or used by others in this country, or patented or described in a printed publication in this or a foreign country, before the invention thereof by the applicant for a patent.
>
> (b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

4. Claims 1, 11, 124, 129-131, 137-141, 143-147, and 155-157 are rejected under 35 U.S.C. 102(a) as being anticipated by Lee et al. (Molecular Cell (June 1998) 1: 1001-1010; newly cited).

These claims are drawn to a method for isothermally amplifying DNA in the absence of exogenously added primers.

Regarding claims 1, 11, 130, 131, 137, 138, 141, and 156, Lee teaches a method for amplifying a template DNA molecule that comprises amplifying the template DNA molecule

under isothermal conditions in a reaction mixture that comprises the DNA template, T7 DNA polymerase, a helicase from bacteriophage T7, a primase from bacteriophage T7, and a single-stranded DNA binding protein (see Figure 1 legend on page 1002 and page 1008, column 2). The amplification method of Lee does not require the addition of exogenously added primers, and the template DNA molecule does not have a terminal protein covalently attached to either 5' end (see Figure 1 legend on page 1002). Lee further teaches that the amount of amplified product produced by the method is at least 10-fold greater than the amount of template put into the reaction mixture (page 1003, column 1).

Regarding claims 124 and 157, Lee teaches that the amount of amplified product generated by the method is at least 100-fold greater than the amount of template put into the reaction mixture (page 1003, column 1).

Regarding claim 129, Lee teaches conducting the method using two forms of T7 DNA polymerase in combination (see Figure 4 and page 1009, column 1).

Regarding claims 139 and 140, Lee teaches that the reaction mixture used to practice the method of claim 1 contains the 63-kDa form of the helicase/primase from bacteriophage T7 (Figure 1 legend and page 1008, column 2).

Regarding claims 143-147, Lee teaches that the amplification is conducted at 30°C, which is less than 60°C, less than 50°C, less than 45°C, less than 40°C, and about 37°C (see Figure 1 legend and page 1009, column 2).

Regarding claim 155, Lee teaches that the amplification reaction used to practice the method of claim 1 further includes potassium glutamate (page 1008, column 1).

5. Claims 1, 11, 124, 137, 138, 141-147, 156, and 157 are rejected under 35 U.S.C. 102(b) as being anticipated by Yuzhakov et al. (Cell (1996) 86: 877-886; newly cited) as evidenced by Bochkarev et al. (Cell (1996) 84: 791-800; newly cited).

These claims are drawn to a method for isothermally amplifying DNA in the absence of exogenously added primers.

Regarding claims 1, 11, 137, 138, 141, 142, and 156, Yuzhakov teaches a method for amplifying a template DNA molecule that comprises amplifying the template DNA molecule under isothermal conditions in a reaction mixture that comprises the DNA template, a DNA polymerase, a helicase, a primase, and an *E. coli* single-stranded DNA binding protein (see page 885, column 2). The amplification method of Yuzhakov does not require the addition of exogenously added primers (see page 885, column 2), and the template DNA molecule does not have a terminal protein covalently attached to either 5' end (see pages 792-796 and Figure 4 of Bochkarev, which teach that the EBNA1 protein binds non-covalently to the 5' end DNA). Yuzhakov further teaches that the amount of amplified product produced by the method is at least 10-fold greater than the amount of template put into the reaction mixture (see Figure 6A and page 885, column 2).

Regarding claims 124 and 157, Yuzhakov teaches that the amount of amplified product generated by the method is at least 100-fold greater than the amount of template put into the reaction mixture (see Figure 6A and page 885, column 2).

Regarding claims 143-147, Yuzhakov teaches that the amplification is conducted at 30°C, which is less than 60°C, less than 50°C, less than 45°C, less than 40°C, and about 37°C (see page 885, column 2).

Claim Rejections - 35 USC § 103

6. The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(e), (f) or (g) prior art under 35 U.S.C. 103(a).

7. Claims 1, 11, 129-139, 141-147, 156, 165, and 167 are rejected under 35 U.S.C. 103(a) as being unpatentable over Scherzinger et al. (European Journal of Biochemistry (1977) 72: 543-558; cited previously) in view of Sorge et al. (US 5,556,772; cited previously) and further in view of Tabor et al. (The Journal of Biological Chemistry (1989) 264(11): 6447-6458; cited previously).

Scherzinger teaches a method for isothermal DNA amplification in the absence of exogenous nucleic acid primers (page 546, column 1).

Regarding claims 1, 11, 130, 131, 137-139, 141, 156, 165, and 167 the method of Scherzinger comprises amplifying a template DNA molecule under isothermal conditions in a

reaction mixture that comprises the DNA template, T7 DNA polymerase, a helicase from bacteriophage T7, a primase from bacteriophage T7, and a single-stranded DNA binding protein (see page 546, column 1). The amplification method of Scherzinger does not require the addition of exogenously added primers, and the template DNA molecule does not have a terminal protein covalently attached to either 5' end. Scherzinger teaches that the amount of amplified product produced by the method is as much as 4-fold greater than the amount of template put into the reaction mixture (page 549, column 2).

Regarding claim 142, Scherzinger teaches that the above method can be performed using an *E. coli* single-stranded DNA binding protein (page 549, column 2).

Regarding claims 143-147, Scherzinger teaches that the amplification is conducted at 30°C, which is less than 60°C, less than 50°C, less than 45°C, less than 40°C, and about 37°C (see page 546, column 1).

Scherzinger does not teach that the yield of amplified product is at least 10-fold greater than the amount of template DNA present at the start of the reaction.

Sorge teaches a composition which comprises a DNA polymerase with substantial 3'-5' exonuclease activity and DNA polymerase modified to have reduced 3' to 5' exonuclease activity (column 2, lines 63-66). Sorge further teaches that wild-type T7 DNA possesses substantial 3' to 5' exonuclease activity (column 3, lines 44-47), whereas modified T7 DNA polymerase has reduced 3' to 5' exonuclease activity (column 4, lines 5-10). Regarding claims 1, 11, 129, 132, 165, and 167, Sorge teaches that conducting amplification reactions in the presence of a DNA polymerase that possesses substantial 3' to 5' exonuclease activity and a DNA polymerase substantially lacking in 3' to 5' exonuclease activity, "increases synthesis product

yield, increased transcription product length, and the synthesis of polynucleotides that can not be synthesized by a given polymerase alone (column 3, lines 22-27)."

Regarding claims 135 and 136, Sorge teaches that the ratio of the two polymerases should be optimized by routine experimentation (column 4, lines 45-58) and further teaches compositions wherein the ratio of the polymerase lacking 3' to 5' exonuclease activity to the polymerase possessing 3' to 5' exonuclease activity is 10:1 (see Table in column 14).

Tabor teaches wild-type and variant forms of T7 DNA polymerase (see abstract). Regarding claims 133 and 134, Tabor teaches that the wild-type T7 DNA polymerase has about 5,000 units of exonuclease activity per milligram of protein, whereas the variant form has less than 50% of the wild-type exonuclease activity (see Table IV on page 6455).

It would have been prima facie obvious to one of ordinary skill in the art at the time of invention to further include a T7 DNA polymerase substantially lacking in 3' to 5' exonuclease activity in the reaction mixture taught by Scherzinger. An ordinary artisan would have been motivated to do so in order to provide an improved system for in vitro DNA synthesis, since Sorge stated that amplification in the presence of a DNA polymerase that possesses substantial 3' to 5' exonuclease activity and a DNA polymerase substantially lacking in 3' to 5' exonuclease activity, "increases synthesis product yield, increased transcription product length, and the synthesis of polynucleotides that can not be synthesized by a given polymerase alone (column 3, lines 22-27)." Finally, an ordinary artisan also would have been motivated to optimize the ratio of the two polymerases to maximize the reaction yield (e.g. to generate at least 10-fold amplification) with a reasonable expectation of success, since Sorge expressly taught that the

concentrations of the two polymerases should be optimized by routine experimentation (column 4, lines 45-58). Moreover, as noted *In re Aller*, 105 USPQ 233 at 235:

> More particularly, where the general conditions of a claim are disclosed in the prior art, it is not inventive to discover the optimum or workable ranges by routine experimentation.

Routine optimization is not considered inventive and no evidence has been presented that the selection of the claimed polymerase ratios was other than routine or that the results should be considered unexpected in any way as compared to the closest prior art.

Finally, regarding the claimed amplification yield of at least 10-fold, attention is also directed to *KSR Int'l Co. v. Teleflex Inc.* (550 U.S.____, 127 S. Ct. 1727 (2007)) where the Supreme Court determined that "a person of ordinary skill has good reason to pursue the known options within his or her technical grasp. If this leads to the anticipated success, it is likely the product not of innovation but of ordinary skill and common sense. In that instance the fact that a combination was obvious to try might show that it was obvious under § 103 (*KSR*, 550 U.S. at ____, 82 USPQ2d at 1397)."

In this case, as discussed above, Scherzinger taught an isothermal, primer-independent DNA amplification method that resulted in 4-fold amplification of the target nucleic acid. Since Sorge taught that the use of a T7 DNA polymerase possessing 3' to 5' exonuclease activity in combination with a T7 DNA polymerase substantially lacking 3' to 5' exonuclease activity, such as the modified T7 DNA polymerase taught by Tabor, improved the yield of DNA synthesis reactions (column 3, lines 22-27), an ordinary artisan would have been motivated to use such a combination of polymerases in the method of Scherzinger to improve the yield of the DNA

synthesis reaction. An ordinary artisan would have been motivated to optimize results-effective variables such as polymerase concentrations, salt concentrations, pH, and temperature by routine experimentation in order to maximize the reaction yield. These methods of improving the yield of *in vitro* DNA amplification reactions were within the technical grasp of the ordinary artisan, and their application to the method resulting from the combined teachings of Scherzinger and Sorge would lead to the anticipated success. Thus, the methods of claims 1, 11, 129-139, 141-147, 156, 165, and 167 are prima facie obvious in view of the combined teachings of Scherzinger, Sorge, and Tabor in the absence of secondary considerations.

8. Claims 24 and 160 are rejected under 35 U.S.C. 103(a) as being unpatentable over Scherzinger et al. (European Journal of Biochemistry (1977) 72: 543-558; cited previously) in view of Sorge et al. (US 5,556,772; cited previously) and further in view of Bernstein et al. (Proceedings of the National Academy of Sciences, USA (1988) 85: 396-400; cited previously).

Regarding claim 24, Scherzinger teaches a method for amplifying a DNA molecule comprising amplifying a template DNA molecule under isothermal conditions in a reaction mixture that comprises the DNA template, wild-type T7 DNA polymerase, a helicase from bacteriophage T7, a primase from bacteriophage T7, and a single-stranded DNA binding protein (see page 546, column 1). The amplification method of Scherzinger does not require the addition of exogenously added primers, and the template DNA molecule does not have a terminal protein covalently attached to either 5' end. Scherzinger teaches that the amount of amplified product produced by the method is as much as 4-fold greater than the amount of template put into the reaction mixture (page 549, column 2).

Scherzinger does not teach that the reaction further comprises a variant T7 DNA polymerase modified to possess reduced 3' to 5' exonuclease activity as required by claim 24. Also, Scherzinger teaches using the 66-kDa or 58-kDa form of the T7 gene 4 protein rather than the 63-kDa form required by claim 24. Finally, Scherzinger does not teach that the yield of amplified product is at least 10-fold greater than the amount of template DNA present at the start of the reaction as required by claim 160.

Sorge teaches a composition which comprises a DNA polymerase with substantial 3'-5' exonuclease activity and a DNA polymerase modified to have reduced 3' to 5' exonuclease activity (column 2, lines 63-66). Sorge further teaches that wild-type T7 DNA polymerase possesses substantial 3' to 5' exonuclease activity (column 3, lines 44-47), whereas modified T7 DNA polymerase has reduced 3' to 5' exonuclease activity (column 4, lines 5-10). Regarding claims 24 and 160, Sorge teaches that conducting amplification reactions in the presence of a DNA polymerase that possesses substantial 3' to 5' exonuclease activity and a DNA polymerase substantially lacking in 3' to 5' exonuclease activity, "increases synthesis product yield, increased transcription product length, and the synthesis of polynucleotides that can not be synthesized by a given polymerase alone (column 3, lines 22-27)."

Bernstein compared the 56 kDa and 63 kDa forms of the T7 gene 4 protein (see abstract). Regarding claim 24, Bernstein teaches that the 7 kDa region absent from the 56 kDa form of the gene 4 protein is required for primase activity (see abstract and page 398).

It would have been prima facie obvious to one of ordinary skill in the art at the time of invention to apply the teachings of Sorge and Bernstein to the method taught by Scherzinger. An ordinary artisan would have been motivated to further include a T7 DNA polymerase

substantially lacking in 3' to 5' exonuclease activity in the reaction mixture taught by Scherzinger, since Sorge stated that amplification in the presence of a DNA polymerase that possesses substantial 3' to 5' exonuclease activity and a DNA polymerase substantially lacking in 3' to 5' exonuclease activity, "increases synthesis product yield, increased transcription product length, and the synthesis of polynucleotides that can not be synthesized by a given polymerase alone (column 3, lines 22-27)." An ordinary artisan also would have been motivated to utilize the 63-kDa form of the T7 gene 4 protein in the method taught by Scherzinger, since Bernstein taught that this protein contained the 7 kDa region critical for primase activity (see abstract and page 398). Finally, an ordinary artisan also would have been motivated to optimize the ratio of the two DNA polymerases in order to maximize the reaction yield (e.g. to generate at least 10-fold amplification) with a reasonable expectation of success, since Sorge expressly taught that the concentrations of the two polymerases should be optimized by routine experimentation (column 4, lines 45-58). As noted in *In re Aller*, 105 USPQ 233 at 235, "Where the general conditions of a claim are disclosed in the prior art, it is not inventive to discover the optimum or workable ranges by routine experimentation." Routine optimization is not considered inventive and no evidence has been presented that the selection of the claimed polymerase ratios was other than routine or that the results should be considered unexpected in any way as compared to the closest prior art.

Finally, regarding the claimed amplification yield of at least 10-fold, attention is also directed to *KSR Int'l Co. v. Teleflex Inc.* (550 U.S.____, 127 S. Ct. 1727 (2007)) where the Supreme Court determined that "a person of ordinary skill has good reason to pursue the known options within his or her technical grasp. If this leads to the anticipated success, it is

likely the product not of innovation but of ordinary skill and common sense. In that instance the fact that a combination was obvious to try might show that it was obvious under § 103 (*KSR*, 550 U.S. at ____, 82 USPQ2d at 1397)."

In this case, as discussed above, Scherzinger taught an isothermal, primer-independent DNA amplification method that resulted in 4-fold amplification of the target nucleic acid. Since Sorge taught that the use of a T7 DNA polymerase possessing 3' to 5' exonuclease activity in combination with a T7 DNA polymerase substantially lacking 3' to 5' exonuclease activity improved the yield of DNA synthesis reactions (column 3, lines 22-27), an ordinary artisan would have been motivated to use such a combination of polymerases in the method of Scherzinger to improve the yield of the DNA synthesis reaction. An ordinary artisan would have been motivated to optimize results-effective variables such as polymerase concentrations, salt concentrations, pH, and temperature by routine experimentation in order to maximize the reaction yield. These methods of improving the yield of in vitro DNA amplification reactions were within the technical grasp of the ordinary artisan, and their application to the method resulting from the combined teachings of Scherzinger and Sorge would lead to the anticipated success. Thus, the methods of claims 24 and 160 are prima facie obvious in view of the combined teachings of Scherzinger, Sorge, and Bernstein in the absence of secondary considerations.

9. Claims 125-128, 157-159, 166, and 168 are rejected under 35 U.S.C. 103(a) as being unpatentable over Scherzinger et al. (European Journal of Biochemistry (1977) 72: 543-558; cited previously) in view of Sorge et al. (US 5,556,772; cited previously) and further in view of

Tabor et al. (The Journal of Biological Chemistry (1989) 264(11): 6447-6458; cited previously) and further in view of Walker et al. (Nucleic Acids Research (1992) 20(7): 1691-1696; newly cited).

The combined teachings of Scherzinger, Sorge, and Tabor result in the method of claims 1, 11, 129-139, 141-147, 156, 165, and 167, as discussed above.

Regarding claims 166 and 168, Scherzinger teaches a method for amplifying a DNA molecule comprising amplifying a template DNA molecule under isothermal conditions in a reaction mixture that comprises the DNA template, wild-type T7 DNA polymerase, a helicase from bacteriophage T7, a primase from bacteriophage T7, and a single-stranded DNA binding protein (see page 546, column 1). The amplification method of Scherzinger does not require the addition of exogenously added primers, and the template DNA molecule does not have a terminal protein covalently attached to either 5' end. Scherzinger teaches that the amount of amplified product produced by the method is as much as 4-fold greater than the amount of template put into the reaction mixture (page 549, column 2).

Regarding claims 125-127, 157, and 158, Scherzinger does not teach 100-10,000,000-fold amplification.

Regarding claims 128, 159, 166, and 168, Scherzinger does not teach exponential amplification.

Walker teaches an isothermal exponential DNA amplification method. The method of Walker utilizes a nicking enzyme to repeatedly generate substrates for a strand displacing DNA polymerase, and thereby, permit isothermal amplification (see abstract, pages 1691-1693, and Figures 1-2). Regarding claims 125-127, 157, and 158, Walker teaches that the method permits

10^7-fold amplification (see abstract and Table 1). Regarding claims 128, 159, 166, and 168, since the nicking enzyme nicks both strands of the double-stranded nucleic acid template, the method permits exponential amplification by the strand displacing DNA polymerase (see Fig. 2).

It would have been prima facie obvious for one of ordinary skill in the art at the time of invention to apply the teachings of Walker to the method resulting from the combined teachings of Scherzinger, Sorge, and Tabor. An ordinary artisan would have been motivated to include a nicking enzyme in the reaction mixture of Scherzinger, since Walker taught that the use of an enzyme that nicked both strands of a double-stranded nucleic acid was useful for separating these double-stranded products of nucleic acid amplification reactions, thereby permitting exponential and isothermal amplification. An ordinary artisan would have recognized that inclusion of a nicking enzyme in the reaction mixture of Scherzinger would have improved the method by permitting exponential rather than linear amplification. An ordinary artisan would have had a reasonable expectation of success in using a nicking enyzme in the reaction mixture of Scherzinger, since nicking enzymes were well known in the art, and Tabor taught that the 3' to 5' exonuclease-deficient T7 DNA polymerase was capable of initiating strand-displacement synthesis at nicks (page 6447, column 2). Thus, the methods of claims 125-128, 157-159, 166, and 168 are prima facie obvious in view of the combined teachings of Scherzinger, Sorge, Tabor, and Walker.

10. Claim 140 is rejected under 35 U.S.C. 103(a) as being unpatentable over Scherzinger et al. (European Journal of Biochemistry (1977) 72: 543-558; cited previously) in view of Sorge et al. (US 5,556,772; cited previously) and further in view of Tabor et al. (The Journal of Biological

Chemistry (1989) 264(11): 6447-6458; cited previously) and further in view of Bernstein et al. (Proceedings of the National Academy of Sciences, USA (1988) 85: 396-400; cited previously).

The combined teachings of Scherzinger, Sorge, and Tabor result in the method of claims 1, 11, 129-139, 141-147, 156, 165, and 167, as discussed above.

Scherzinger teaches using the 66-kDa or 58-kDa form of the T7 gene 4 protein rather than the 63-kDa form required by claim 140.

Bernstein compared the 56 kDa and 63 kDa forms of the T7 gene 4 protein (see abstract). Regarding claim 140, Bernstein teaches that the 7 kDa region absent from the 56 kDa form of the gene 4 protein is required for primase activity (see abstract and page 398).

It would have been prima facie obvious for one of ordinary skill in the art at the time of invention to apply the teachings of Bernstein to the method resulting from the combined teachings of Scherzinger, Sorge, and Tabor. An ordinary artisan would have been motivated to utilize the 63-kDa form of the T7 gene 4 protein in the reaction mixture taught by Scherzinger, since Bernstein taught that this protein contained the 7 kDa region critical for primase activity (see abstract and page 398). An ordinary artisan would have had a reasonable expectation of success in doing so, since Bernstein taught a method for purifying this protein (page 396, column 1). Thus, the method of claim 140 is prima facie obvious in view of the combined teachings of Scherzinger, Sorge, Tabor, and Bernstein.

11. Claims 148 and 149 are rejected under 35 U.S.C. 103(a) as being unpatentable over Scherzinger et al. (European Journal of Biochemistry (1977) 72: 543-558; cited previously) in view of Sorge et al. (US 5,556,772; cited previously) and further in view of Tabor et al. (The

Journal of Biological Chemistry (1989) 264(11): 6447-6458; cited previously) and further in view of Dickinson et al. (Journal of Cell Science (1983) 60: 355-365; cited previously).

The combined teachings of Scherzinger, Sorge, and Tabor result in the method of claims 1, 11, 129-139, 141-147, 156, 165, and 167, as discussed above.

These references do not teach including a nucleotide diphosphokinase in the amplification reaction.

Dickinson teaches the nucleotide diphosphokinase is required for DNA replication in yeast (see summary on page 355).

It would have been prima facie obvious for one of ordinary skill in the art at the time of invention to include a nucleotide diphosphokinase in the reaction mixture of Scherzinger. Since Dickinson taught that this enzyme was required for DNA replication, an ordinary artisan would have been motivated to further include this enzyme in the reaction mixture of Scherzinger in order to improve the ability of the *in vitro* system to synthesize DNA. Since purified forms of this enzyme were commercially available at the time of invention, an ordinary artisan would have had a reasonable expectation of success in incorporating this essential enzyme into the reaction mixture of Scherzinger. Thus, the methods of claims 148 and 149 are prima facie obvious in view of the combined teachings of Scherzinger, Sorge, Tabor, and Dickinson.

12. Claims 148 and 150 are rejected under 35 U.S.C. 103(a) as being unpatentable over Scherzinger et al. (European Journal of Biochemistry (1977) 72: 543-558; cited previously) in view of Sorge et al. (US 5,556,772; cited previously) and further in view of Tabor et al. (The

Journal of Biological Chemistry (1989) 264(11): 6447-6458; cited previously) and further in view of Peller (Biochemistry (1977) 16(3): 387-395; cited previously).

The combined teachings of Scherzinger, Sorge, and Tabor result in the method of claims 1, 11, 129-139, 141-147, 156, 165, and 167, as discussed above.

These references do not teach including an inorganic pyrophosphatase in the amplification reaction.

Peller investigated the role of pyrophosphate hydrolysis during in vitro synthesis of bacteriophage T7 DNA (see abstract). Peller sought to develop a thermodynamic explanation for the observation that nucleic acids generated by cell-free in vitro synthesis systems often produced products at the small end of the size spectrum (page 387, column 1). Peller stated, "The achievement of both very high molecular weights and sharply peaked size distributions in polynucleotides synthesized in vitro will require coupling to inorganic pyrophosphatase action as in vivo (see abstract)."

It would have been prima facie obvious for one of ordinary skill in the art at the time of invention to include an inorganic pyrophosphatase in the isothermal amplification reaction mixture taught by Scherzinger. As noted above, Peller expressly taught that synthesis of nucleic acids of high molecular weight required inorganic pyrophosphatase activity (see abstract). An ordinary artisan would have been motivated by these teachings of Peller to further include this enzyme in the reaction mixture of Scherzinger in order to improve the ability of the *in vitro* system to synthesize high molecular weight DNA. Since purified forms of this enzyme were commercially available at the time of invention, an ordinary artisan would have had a reasonable expectation of success in incorporating this essential enzyme into the reaction mixture of

Scherzinger. Thus, the method of claims 148 and 150 are prima facie obvious in view of the combined teachings of Scherzinger, Sorge, Tabor, and Peller.

13. Claims 148, 151 and 152 are rejected under 35 U.S.C. 103(a) as being unpatentable over Scherzinger et al. (European Journal of Biochemistry (1977) 72: 543-558; cited previously) in view of Sorge et al. (US 5,556,772; cited previously) and further in view of Tabor et al. (The Journal of Biological Chemistry (1989) 264(11): 6447-6458; cited previously) and further in view of Nakai et al. (The Journal of Biological Chemistry (1993) 268(32): 23997-24004; cited previously).

The combined teachings of Scherzinger, Sorge, and Tabor result in the method of claims 1, 11, 129-139, 141-147, 156, 165, and 167, as discussed above.

These references do not teach including an ATP regeneration system comprising creatine kinase and phosphocreatine in the amplification reaction.

Nakai teaches methods of conducting in vitro DNA synthesis reactions using the T7 replication system (page 23998, column 1). Regarding claims 148, 151, and 152, Nakai teaches that the reaction mixture includes an ATP regenerating system that includes phosphocreatine and creatine kinase (page 23998, column 1).

It would have been prima facie obvious for one of ordinary skill in the art at the time of invention to include an ATP regeneration system comprising creatine kinase and phosphocreatine in the isothermal amplification reaction mixture taught by Scherzinger. As noted above, Nakai taught conducting in vitro DNA synthesis reactions using the T7 replication system in the presence of an ATP regeneration system comprising creatine kinase and

phosphocreatine (page 23998, column 1). An ordinary artisan would have been motivated by these teachings of Nakai to further include the ATP regeneration system in the reaction mixture of Scherzinger in order to improve the ability of the *in vitro* system to synthesize DNA. Since creatine kinase and phosphocreatine were commercially available at the time of invention, an ordinary artisan would have had a reasonable expectation of success in incorporating an ATP regeneration system into the reaction mixture of Scherzinger. Thus, the method of claims 148, 151, and 152 are prima facie obvious in view of the combined teachings of Scherzinger, Sorge, Tabor, and Nakai.

14. Claims 153 and 154 are rejected under 35 U.S.C. 103(a) as being unpatentable over Scherzinger et al. (European Journal of Biochemistry (1977) 72: 543-558; cited previously) in view of Sorge et al. (US 5,556,772; cited previously) and further in view of Tabor et al. (The Journal of Biological Chemistry (1989) 264(11): 6447-6458; cited previously) and further in view of Engler et al. (The Journal of Biological Chemistry (1983) 258(18): 11197-11205; cited previously).

The combined teachings of Scherzinger, Sorge, and Tabor result in the method of claims 1, 11, 129-139, 141-147, 156, 165, and 167, as discussed above.

These references do not teach including T7 DNA ligase in the amplification reaction.

Engler teaches a method for conducting lagging strand synthesis using an *in vitro* T7 replication system (see abstract). Engler teaches that T7 DNA ligase activity is required for successful lagging strand synthesis (see abstract). Engler stated, "All steps in the replication of a lagging strand have been coupled in a model system that catalyzes the formation of covalently

closed, circular, double-stranded DNA molecules using single-stranded viral DNA as template. A combination of four bacteriophage proteins, gene 4 protein, Form II of T7 DNA polymerase, gene 6 exonuclease, and DNA ligase, can accomplish this overall reaction (abstract)."

It would have been prima facie obvious for one of ordinary skill in the art at the time of invention to include T7 DNA ligase in the isothermal amplification reaction mixture of Scherzinger. As noted above, Engler expressly taught that DNA ligase was required for lagging strand synthesis during *in vitro* replication (see above). An ordinary artisan would have been motivated by these teachings of Engler to include T7 DNA ligase in the reaction mixture of Scherzinger in order to obtain the ability to generate closed circular double-stranded DNA molecules from the closed circular single-stranded DNA template used by Scherzinger. Since Engler taught a method for purifying T7 DNA ligase (page 11198, column 2), an ordinary artisan would have had a reasonable expectation of success in adding this enzyme to the reaction mixture taught by Scherzinger. Thus, the methods of claims 153 and 154 are prima facie obvious in view of the combined teachings of Scherzinger, Sorge, Tabor, and Engler.

15. Claim 155 is rejected under 35 U.S.C. 103(a) as being unpatentable over Scherzinger et al. (European Journal of Biochemistry (1977) 72: 543-558; cited previously) in view of Sorge et al. (US 5,556,772; cited previously) and further in view of Tabor et al. (The Journal of Biological Chemistry (1989) 264(11): 6447-6458; cited previously) and further in view of in view of Jarvis et al. (The Journal of Biological Chemistry (1990) 265(25): 15160-15167; cited previously).

The combined teachings of Scherzinger, Sorge, and Tabor result in the method of claims 1, 11, 129-139, 141-147, 156, 165, and 167, as discussed above.

These references do not teach that the reaction mixture further includes an additive selected from the group consisting of dextran, potassium glutamate, and DMSO.

Jarvis studied the effect of including high molecular weight polymers such as dextran or polyethylene glycol (PEG) on the stability and processivity of the T4 DNA replication complex (abstract and page 15161). Jarvis reported that PEG and dextran stabilized the DNA replication complex, and thereby, indirectly increased processivity of the polymerase (see Table 1 on page 15162). Jarvis stated, "Although the processivity of the polymerase alone is not directly effected by the addition of such polymers to the solution, macromolecular crowding does significantly stabilize the holoenzyme and thus indirectly increases the observed processivity of the holoenzyme complex (abstract)." Jarvis further stated, "These results suggest that the volume-occupied solution conditions prevalent in vivo can significantly stabilize holoenzyme DNA replication complexes, and thus support high rates and high apparent processivities of DNA synthesis (page 15166)."

It would have been prima facie obvious for one of ordinary skill in the art at the time of invention to include dextran in the isothermal amplification reaction mixture taught by Scherzinger. As noted above, Jarvis expressly taught that dextran stabilized the T4 DNA replication complex, and thereby, increased processivity of the polymerase. An ordinary artisan would have been motivated by these teachings of Jarvis to include dextran in the reaction mixture taught by Scherzinger in order to stabilize the T7 DNA replication complex, and thereby, improve polymerase processivity with the ultimate result being increased amplification efficiency and yield. An ordinary artisan would have expected a reasonable level of success in doing so, since the Jarvis observed increased processivity in the highly similar T4 replication

system and further taught that that the crowding effect responsible for the observed stabilization/increased processivity was generally applicable (pages 15161 and 15166). Thus, the method of claim 155 is prima facie obvious in view of the combined teachings of Scherzinger, Sorge, Tabor, and Jarvis.

16. Claims 161-164 and 169 are as being unpatentable over Scherzinger et al. (European Journal of Biochemistry (1977) 72: 543-558; cited previously) in view of Sorge et al. (US 5,556,772; cited previously) and further in view of Bernstein et al. (Proceedings of the National Academy of Sciences, USA (1988) 85: 396-400; cited previously) and further in view of Walker et al. (Nucleic Acids Research (1992) 20(7): 1691-1696; newly cited) and further in view of Tabor et al. (The Journal of Biological Chemistry (1989) 264(11): 6447-6458; cited previously).

The combined teachings of Scherziner, Sorge, and Bernstein result in the method of claims 24 and 160, as discussed above.

Regarding claim 169, Scherzinger teaches a method for amplifying a DNA molecule comprising amplifying a template DNA molecule under isothermal conditions in a reaction mixture that comprises the DNA template, wild-type T7 DNA polymerase, a helicase from bacteriophage T7, a primase from bacteriophage T7, and a single-stranded DNA binding protein (see page 546, column 1). The amplification method of Scherzinger does not require the addition of exogenously added primers, and the template DNA molecule does not have a terminal protein covalently attached to either 5' end. Scherzinger teaches that the amount of amplified product produced by the method is as much as 4-fold greater than the amount of template put into the reaction mixture (page 549, column 2).

Scherzinger does not teach 100-10,000,000-fold amplification as required by claims 161-163. Scherzinger also does not teach that the reaction further comprises a variant T7 DNA polymerase modified to possess reduced 3' to 5' exonuclease activity as required by claim 169. Scherzinger teaches using the 66-kDa or 58-kDa form of the T7 gene 4 protein rather than the 63-kDa form required by claim 169. Finally, Scherzinger does not teach exponential amplification as required by claims 164 and 169.

Sorge teaches a composition which comprises a DNA polymerase with substantial 3'-5' exonuclease activity and DNA polymerase modified to have reduced 3' to 5' exonuclease activity (column 2, lines 63-66). Sorge further teaches that wild-type T7 DNA possesses substantial 3' to 5' exonuclease activity (column 3, lines 44-47), whereas modified T7 DNA polymerase has reduced 3' to 5' exonuclease activity (column 4, lines 5-10). Regarding claim 169, Sorge teaches that conducting amplification reactions in the presence of a DNA polymerase that possesses substantial 3' to 5' exonuclease activity and a DNA polymerase substantially lacking in 3' to 5' exonuclease activity, "increases synthesis product yield, increased transcription product length, and the synthesis of polynucleotides that can not be synthesized by a given polymerase alone (column 3, lines 22-27)."

Bernstein compared the 56 kDa and 63 kDa forms of the T7 gene 4 protein (see abstract). Regarding claim 169, Bernstein teaches that the 7 kDa region absent from the 56 kDa form of the gene 4 protein is required for primase activity (see abstract and page 398).

Walker teaches an isothermal exponential DNA amplification method. The method of Walker utilizes a nicking enzyme to repeatedly generate substrates for a strand displacing DNA polymerase, and thereby permit isothermal amplification (see abstract, pages 1691-1693, and

Figures 1-2). Regarding claims 161-163, Walker teaches that the method permits 10^7-fold amplification (see abstract and Table 1). Regarding claims 164 and 169, since the nicking enzyme nicks both strands of the double-stranded nucleic acid template, the method permits exponential amplification by the strand displacing polymerase (see Figure 2).

Tabor teaches wild-type and variant forms of T7 DNA polymerase (see abstract). Regarding claims 161-164 and 169, Tabor teaches that the modified T7 DNA polymerase that lacks 3' to 5' exonuclease activity is capable of initiating strand-displacement synthesis at nicks (page 6447, column 2).

It would have been prima facie obvious to one of ordinary skill in the art at the time of invention to apply the teachings of Sorge, Bernstein, and Walker to the method taught by Scherzinger. An ordinary artisan would have been motivated to further include a T7 DNA polymerase substantially lacking in 3' to 5' exonuclease activity in the reaction mixture taught by Scherzinger, since Sorge stated that amplification in the presence of a DNA polymerase that possesses substantial 3' to 5' exonuclease activity and a DNA polymerase substantially lacking in 3' to 5' exonuclease activity, "increases synthesis product yield, increased transcription product length, and the synthesis of polynucleotides that can not be synthesized by a given polymerase alone (column 3, lines 22-27)." An ordinary artisan also would have been motivated to utilize the 63-kDa form of the T7 gene 4 protein in the method taught by Scherzinger, since Bernstein taught that this protein contained the 7 kDa region critical for primase activity (see abstract and page 398). Finally, an ordinary artisan would have been motivated to include a nicking enzyme in the reaction mixture of Scherzinger, since Walker taught that the use of an enzyme that nicked both strands of a double-stranded nucleic acid was useful for separating these double-stranded

products of nucleic acid amplification reactions, thereby permitting exponential and isothermal amplification. An ordinary artisan would have recognized that inclusion of a nicking enzyme in the reaction mixture of Scherzinger would have improved the method by permitting exponential rather than linear amplification. An ordinary artisan would have had a reasonable expectation of success in using a nicking enyzme in the reaction mixture of Scherzinger, since nicking enzymes were well known in the art, and Tabor taught that the 3' to 5' exonuclease-deficient T7 DNA polymerase was capable of initiating strand-displacement synthesis at nicks (page 6447, column 2). Thus, the methods of claims 161-164 and 169 are prima facie obvious in view of the combined teachings of Scherzinger, Sorge, Bernstein, Tabor, and Walker.

Response to Arguments

17. As noted above, Applicant's arguments regarding the objection to the specification, the rejection of claims 1, 11, 124-142, and 147-163 under 112, second paragraph, and the rejections under § 102(b) and § 103(a) were persuasive. These rejections have been withdrawn.

It is noted that the arguments do not appear to address the issue of the indefiniteness of the phrase "less than about" appearing in claims 143-146. As discussed previously and reiterated above, the use of the phrase "less than about" renders the upper boundary of the claimed range completely unclear, and therefore, the claims are indefinite. This rejection has been maintained.

Conclusion

No claims are currently allowable.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Angela Bertagna whose telephone number is 571-272-8291. The examiner can normally be reached on M-F, 7:30 - 5.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Gary Benzion can be reached on 571-272-0782. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

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